

# Sill Cas9-KO Strategy

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### Overview

#### **Target Gene Name**

• Sil1

#### **Project Type**

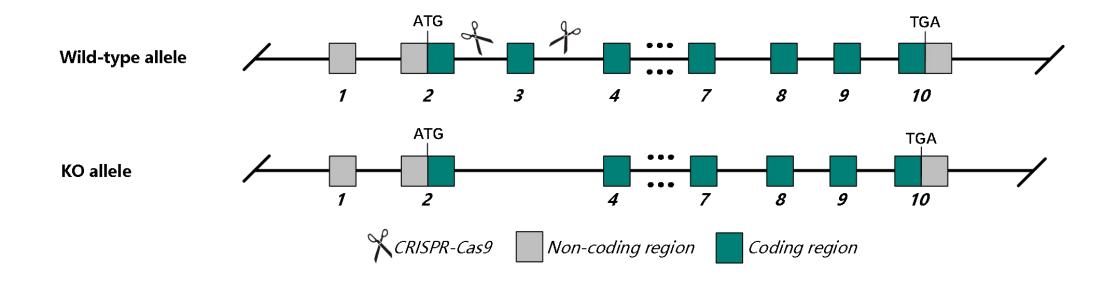
• Cas9-KO

#### Genetic Background

• C57BL/6JGpt



## Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Sil1 gene.



#### **Technical Information**

- The *Sil1* gene has 11 transcripts. According to the structure of *Sil1* gene, exon3 of *Sil1*-201 (ENSMUST00000025215.10) transcript is recommended as the knockout region. The region contains 139bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Sil1* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and ontarget amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



#### Gene Information

#### Sil1 SIL1 nucleotide exchange factor [ Mus musculus (house mouse) ]

Gene ID: 81500, updated on 12-Apr-2023

**≜** Summary

Official Symbol Sil1 provided by MGI

Official Full Name SIL1 nucleotide exchange factor provided by MGI

Primary source MGI:MGI:1932040

See related Ensembl: ENSMUSG00000024357 AllianceGenome: MGI:1932040

Gene type protein coding RefSeg status VALIDATED Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as wz; 1810057E01Rik

Summary Predicted to enable adenyl-nucleotide exchange factor activity and identical protein binding activity. Predicted to act upstream of or within protein transport. Located in endoplasmic reticulum. Is expressed in

brain; chondrocranium; eye; pancreas; and testis. Human ortholog(s) of this gene implicated in primary cerebellar degeneration. Orthologous to human SIL1 (SIL1 nucleotide exchange factor). [provided by

Alliance of Genome Resources, Apr 2022]

Expression Ubiquitous expression in testis adult (RPKM 27.6), adrenal adult (RPKM 12.9) and 28 other tissues See more

Orthologs human all

Try the new Gene table

Try the new Transcript table

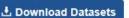
Genomic context

Location: 18: 18 B1- B2 See Sil1 in Genome Data Viewer

Exon count: 15

Source: https://www.ncbi.nlm.nih.gov/









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## Transcript Information

The gene has 11 transcripts, all transcripts are shown below:

| Transcript ID         | Name     | bp 🛊 | Protein      | Translation ID       | Biotype                        | CCDS        | UniProt Match       | Flags   |
|-----------------------|----------|------|--------------|----------------------|--------------------------------|-------------|---------------------|---|
| ENSMUST00000025215.10 | Sil1-201 | 1703 | <u>465aa</u> | ENSMUSP00000025215.9 | Protein coding                 | CCDS29140 ₺ | Q9EPK6₽             | Ensembl Canonical GENCODE basic APPRIS P1 TSL:1 |
| ENSMUST00000235524.2  | Sil1-202 | 335  | 38aa         | ENSMUSP00000157758.2 | Protein coding                 |             | <u>A0A494B9U3</u> ₺ | GENCODE basic                                   |
| ENSMUST00000235619.2  | Sil1-203 | 1524 | <u>417aa</u> | ENSMUSP00000157636.2 | Protein coding                 |             | A0A494B9H1₺         | GENCODE basic                                   |
| ENSMUST00000235691.2  | Sil1-204 | 355  | <u>20aa</u>  | ENSMUSP00000157444.2 | Protein coding                 |             | A0A494B950₺         | CDS 3' incomplete                               |
| ENSMUST00000235778.2  | Sil1-205 | 342  | <u>28aa</u>  | ENSMUSP00000157667.2 | Protein coding                 |             | A0A494B9L8₺         | CDS 3' incomplete                               |
| ENSMUST00000236007.2  | Sil1-206 | 538  | <u>53aa</u>  | ENSMUSP00000157932.2 | Protein coding                 |             | A0A494BA83 ₺        | GENCODE basic                                   |
| ENSMUST00000236387.2  | Sil1-207 | 1532 | No protein   | ((2)                 | Protein coding CDS not defined |             | -                   | (2)   |
| ENSMUST00000237232.2  | Sil1-208 | 453  | <u>80aa</u>  | ENSMUSP00000158247.2 | Protein coding                 |             | <u>A0A494BAV3</u> ₽ | CDS 3' incomplete                               |
| ENSMUST00000237253.2  | Sil1-209 | 570  | No protein   | (5)                  | Protein coding CDS not defined |             | -                   | 650   |
| ENSMUST00000237309.2  | Sil1-210 | 695  | <u>119aa</u> | ENSMUSP00000157506.2 | Protein coding                 |             | A0A494B962₺         | CDS 5' incomplete                               |
| ENSMUST00000237896.2  | Sil1-211 | 1501 | 327aa        | ENSMUSP00000157521.2 | Protein coding                 |             | A0A494B9B2₺         | GENCODE basic                                   |

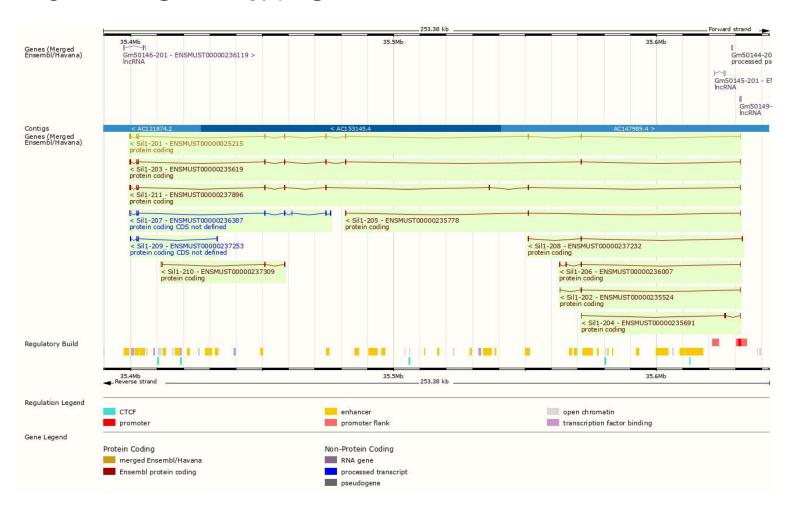
The strategy is based on the design of *Sil1*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



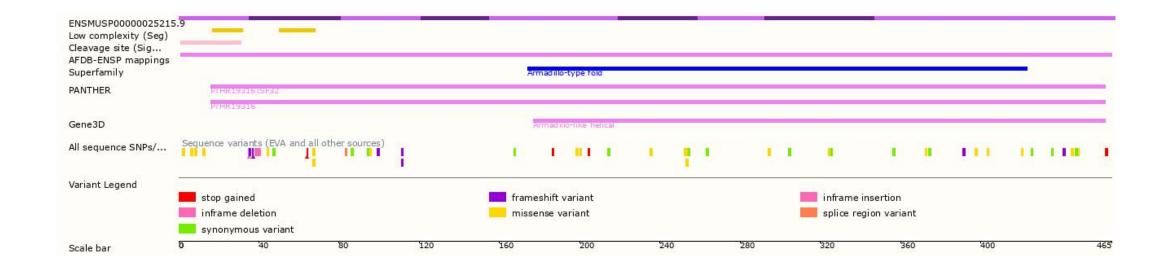
#### Genomic Information





Source: https://www.ensembl.org

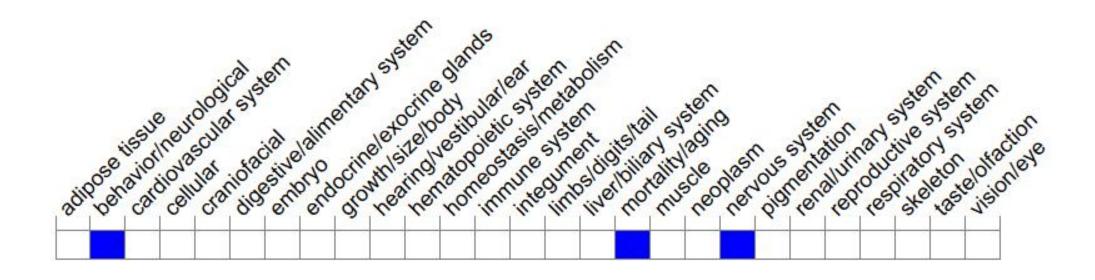
#### Protein Information





Source: : https://www.ensembl.org

## Mouse Phenotype Information (MGI)



• Phenotypes affected by the mutations of *Sil1* gene are marked in blue. Mice homozygous for a gene trapped allele or spontaneous mutation exhibit ataxia and Purkinje cell degeneration.



Source: https://www.informatics.jax.org

## Important Information

- According to the existing MGI data, mice homozygous for a gene trapped allele or spontaneous mutation exhibit ataxia and Purkinje cell degeneration, and altered hemodynamicsn leading to perinatal lethality.
- The effect of this strategy on the transcript of *Sil1*-202(ENSMUST00000235524.2), *Sil1*-203(ENSMUST00000235619.2), *Sil1*-204(ENSMUST00000235691.2), *Sil1*-206(ENSMUST00000236007.2), *Sil1*-207(ENSMUST00000236387.2), *Sil1*-209(ENSMUST00000237253.2) and *Sil1*-210(ENSMUST00000237309.2) is unknown.
- *Sil1* is located on Chr18. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

